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FLOW CYTOMETRIC QUANTIFICATION OF RADIATION RESPONSES OF MURINE PERITONEAL CELLS*

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ASBTRACT

Methods have been developed to distinguish subpopulations of murine peritoneal cells, and these were applied to the measurement of early changes in peritoneal cells after irradiation. The ratio of the two major subpopulations in the peritoneal fluid, lymphocytes and macrophages, was measured rapidly by means of cell volume distribution analysis as well as by hypotonic propidium iodide (PI) staining. After irradiation, dose and time dependent changes were noted in the cell volume distributions: a rapid loss of peritoneal lymphocytes, and an increase in the mean cell volume of macrophages. The hypotonic PI staining characteristics of the peritoneal cells showed two or three distinctive G_1 peaks. The ratio of the areas of these peaks was also found to be dependent on the radiation dose and the time after irradiation. These results demonstrate that these two parameters may be used to monitor changes induced by irradiation (biological dosimetry), and to sort different peritoneal subpopulations.

Key words: Flow cytometry, biological dosimetry, x rays, peritoneal cells, macrophages, lymphocytes

INTRODUCTION

Quantification of radiation response is the basis for biological dosimetry. The conventional dosimeters used for low-LET irradiation have been peripheral lymphocyte counting and chromosome aberration enumeration. More recently, other dosimetric methods have also been reported, e.g., dosimetry based on spermatogenesis (Hacker et al. 1980) and on peripheral reticulocyte counting (Chaudhuri et al. 1979). The study of chromosome aberrations in peripheral lymphocytes provides an average dose estimate within a few days and has been used clinically (Purrot et al. 1972; Doloy et al. 1977).

The development of flow cytometry has made it possible to measure biological parameters rapidly and with a high degree of statistical accuracy. This technology has been applied to the field of biological dosimetry (Carrano et al. 1978; Hacker et al. 1980). In an attempt to shorten the assay period, i.e., obtaining results within 24 hours after exposure, and to make the assay independent of circulating lymphocytes, we have measured the radiation responses of mouse peritoneal cells using flow cytometry. Two major subpopulations, lymphocytes and macrophages, exist in the peritoneal cavity of mice (Felix and Dalton 1955; Goodman 1964; Balner 1963). Several studies have shown that the number of peritoneal lymphocytes was reduced after irradiation, while the macrophage population remained relatively unchanged (Balner 1963; Bercovici and Graham 1964; Kornfeld and Greenman 1966). Because of cell size differences between lymphocytes and macrophages (Felix and Dalton 1955; Kornfeld and Greenman 1966) and our preliminary data indicating different propidium iodide staining characteristics of peritoneal subpopulations, the radiation response of murine peritoneal cells was measured in terms of cell volume distributions and DNA fluorescence staining intensity using flow cytometry with a view to develop a rapid biological dosimetry system.

MATERIALS AND METHODS

Mice (strain CD-1, female, 8-12 weeks old, Charles River Laboratories) were used for this study. Six to ten mice were used per dose point. Whole body irradiation of mice was carried out with x rays (GE Maxitron, 250 KeV, 30 mA, 2 mm Cu HVL, a dose rate of 2 Gy/min) through a single portal. At various times after irradiation, 8 ml of alpha MEM medium (Grand Island Biological Company) containing 5 USP units per ml of sodium heparin and 190 mM morpholinopropane-sulfonic acid (Sigma Chemical Company) at 37°C was injected intraperitoneally. Approximately 15 min after i.p. injection, the mice were sacrificed and the fluid removed from the peritonial cavity. The average number of cells obtained from unirradiated mice was 6-8 x 10^6 cells, while the total number of cells obtained from the peritoneal cavity of irradiated mice decreased depending on the radiation dose and time after irradiation. After pipetting repeatedly, aliquots of the cell suspension were used for cell counting using a hemocytometer or an electronic cell counter (Coulter Electronics). Cell volume distribution analyses were performed with the addition of a pulse height analyzer to the Coulter counter. The remaining cells were centrifuged at 300 g for 5 min. Part of the cells were used for histological staining; the remaining cells were fixed in 70% ethanol for 30 min at 4°C. The ethanol-fixed cells were stained with a hypotonic propidium iodide solution containing 50 µg/ml propidium iodide (Sigma) and 1 mg/ml sodium citrate in distilled water. This is a modification of the staining procedure of Crissman and Steinkamp (1973) and of Krishan (1975). The measurement of PI fluorescence as well as cell sorting were performed on flow cytometers available at Los Alamos using an argon-ion laser operating at 488 nm and the appropriate filters and detectors (Holm and Cram 1973; Steinkamp et al. 1973).

RESULTS

The cell volume distributions of peritoneal cells measured 12 hours after 0 to 11 Gy of x rays are shown in Figure 1. Cells from each peak in the control were sorted onto slides and histological observations demonstrated that the small and large volume populations corresponded to lymphocytes and macrophages respectively. As the radiation dose was increased, the fraction of the cells in the first peak decreased progressively as a result of a rapid loss of peritoneal lymphocytes. The number of macrophages remained unchanged within 24 hrs after irradiation.

Figure 2 shows the cell volume distributions of peritoneal cells measured 24 hours after 0 to 8 Gy of x rays. In addition to the changes in the relative areas of the two peaks, there is a shift of the second peak towards larger cell volumes at 5 and 8 Gy. A cytological examination revealed that the macrophages obtained from mice receiving 8 Gy appeared 20-30% larger than those from unirradiated mice. There were also an increased number of granulocytes in the peritoneal cavity of irradiated mice.

In order to quantitate the changes in peritoneal cell subpopulations after irradiation, the fraction of cells in the larger volume peak was measured by dividing the two populations at their inflection point and calculating the fraction of cells above the inflection point as a percent of the total population. Figure 3 shows the percent of the population contained in the large volume peak plotted as a function of radiation dose. The relative increase in the fraction of cells in the second peak was similar at 12 and 24 hours after irradiation. However, the relative increase in second peak fraction was enhanced at 48 hours.

When ethanol-fixed peritoneal cells were stained with hypotonic PI solution without RNase, two or three peaks were observed in the G_1 fluorescence intensity region. Figure 4 shows the fluorescence intensity of the PI-stained peritoneal cells 24 hrs after irradiation. Both of the two prominent peaks in the control correspond to G_1 fluorescence intensity peaks. Cell sorting of the two populations onto slides followed by microscopic examination indicated that the cells in the lower fluorescence intensity peak appeared to be mostly lymphocytes along with some granulocytes, whereas the cells comprizing the higher fluorescence intensity peak were macrophages with some large lymphocytes. As

the radiation dose was increased, the first peak area also decreased. Figure 5 shows the G_1 fluorescence intensity distributions of PI-stained cells collected 0 to 8 days after a single dose of 5 Gy. The relative second-peak area increased with longer times after irradiation.

The fraction of cells in the higher G_1 fluorescence intensity peak was estimated by the same method described for volume distributions and plotted as a function of radiation dose. Figure 6 shows the dose-response patterns measured at various times after irradiation. There is no significant change noted in the fraction of cells in the lower fluorescence intensity peak when the cells were sampled between 12 to 48 hours after irradiation.

DISCUSSION

There were two types of changes noted in the cell volume distributions of peritoneal cells after irradiation: (1) a shift in the ratio of small to large cells due to a rapid loss of lymphocytes, and (2) an increase in the mean cell volume of macrophages seen 24 hours after irradiation. The rapid loss of peritoneal lymphocytes after irradiation has been well documented (Balner 1963; Bercovici and Graham 1963; Kornfeld and Greenman 1966). The increase in cell volume seen only after higher doses (Figure 2) is significant. Geiger and Gallily (1973) observed numerous invaginations or "holes" on the outer surfaces of irradiated macrophages. It is not known whether the increase in size observed in our study is an artifact caused by suspending the cells in medium. Metcalf et al. (1977) reported that mouse bone marrow progenitors of granulocytes and macrophages increased in size 2 days after 2.5 Gy whole body irradiation and that all subpopulations were affected. In our study, however, the size increase appeared limited to the macrophage population.

The multimodal G_1 fluorescence intensity observed after PI staining of peritoneal cells indicates that staining characteristics differ for various types of cells even though the cells had been fixed in ethanol prior to staining. When the cells obtained at 0-8 days after irradiation (0 to 11 Gy) were stained with the method described by Krishan (1975), in which RNase was also added, no multimodal G_1 fluorescence intensity peaks were observed. The CV value for the G_1 peak was rather high (4-5%), however, and no cells were found with S-phase DNA contents (data not shown). Since the PI solution used in this study stains double-stranded RNA in addition to DNA (Crissman and Steinkamp 1973), the differences in double-stranded RNA content between the two subpopulations might have contributed to the separation of the control G_1 cells into distinct fluorescence intensity peaks. The changes in the relative size of the multimodal G_1 peaks of irradiated peritoneal cells appear to have been caused primarily by alterations in the cell populations. Geiger and Gallily (1974) reported that irradiation of macrophage donors caused activation of several macrophage functions, including a six-fold increase in RNA synthesis. It is yet to be determined if such an increase in RNA synthesis in irradiated macrophages has also affected the multimodal G_1 fluorescence intensity pattern seen in this study.

The results demonstrate that cell volume distributions as well as hypotonic PI staining may be used to monitor changes induced by irradiation (biological dosimetry), and to sort different peritoneal subpopulations.

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FIGURE LEGENDS

Figure 1. Cell volume distributions of murine peritoneal cells obtained 12 hours after irradiation. The cells comprizing the small and large volumes peaks in the control population correspond to lymphocytes and macrophages, respectively.

Figure 2. Cell volume distributions of peritoneal cells obtained 24 hours after 0 to 8 Gy x-irradiation. The cell volume distributions of cells taken after single doses of 5 and 8 Gy show an increase in the mean cell volume of macrophages.

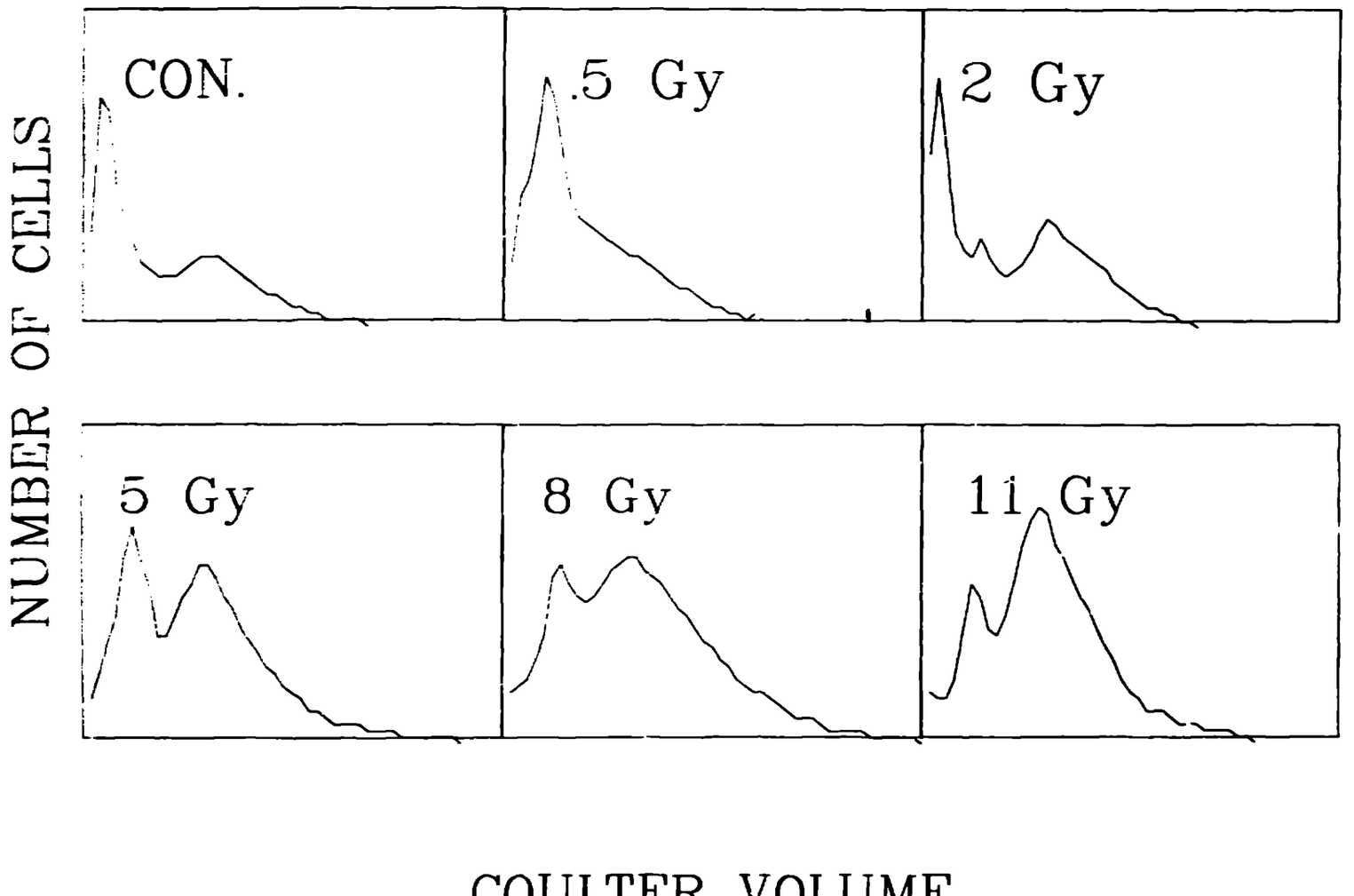
Figure 3. The fraction of cells in the second peak of each cell volume distribution expressed as a percent of the total population plotted as a function of radiation dose.

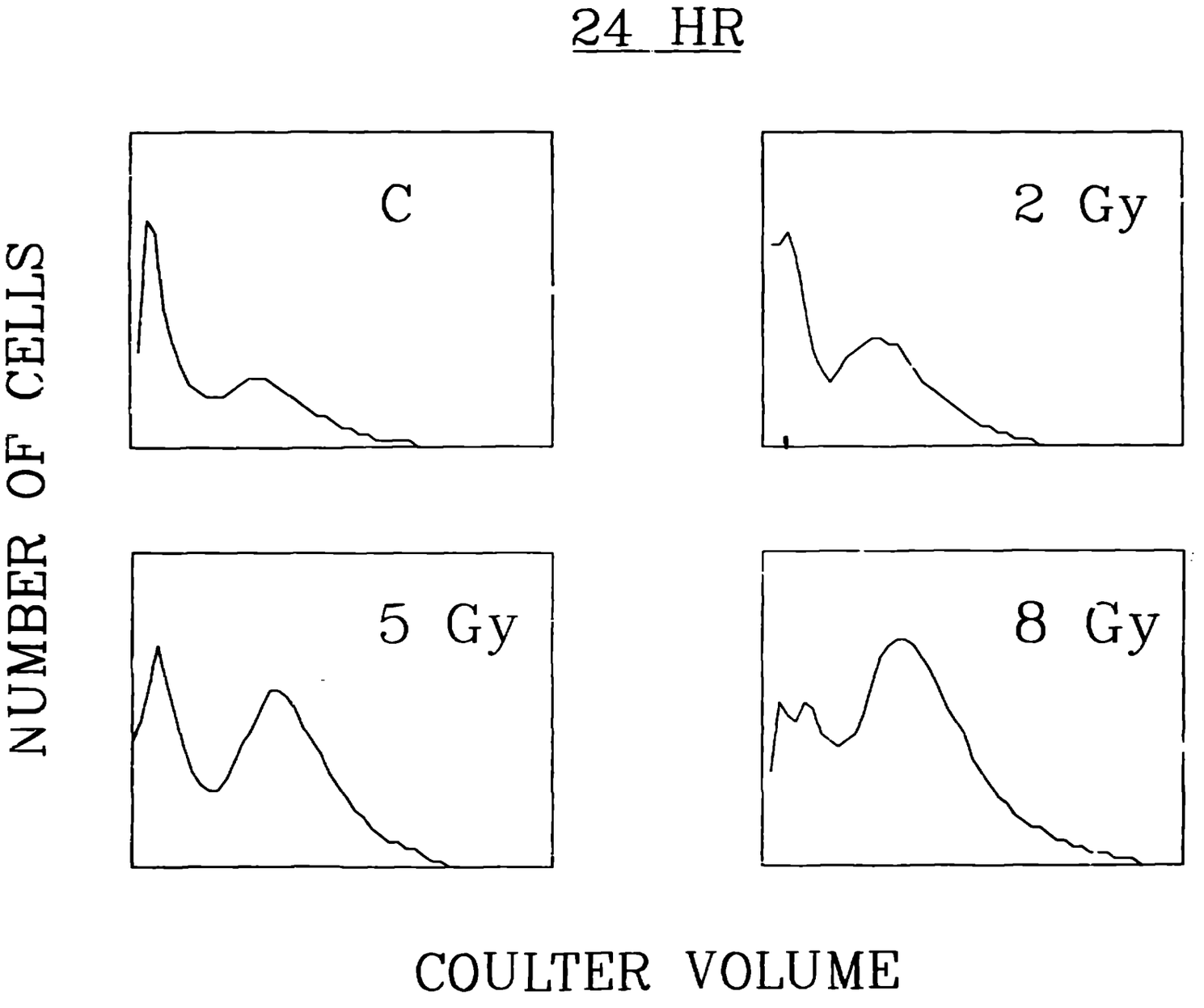
Figure 4. Propidium iodide staining characteristics of G_1 peritoneal cells taken 24 hrs after irradiation.

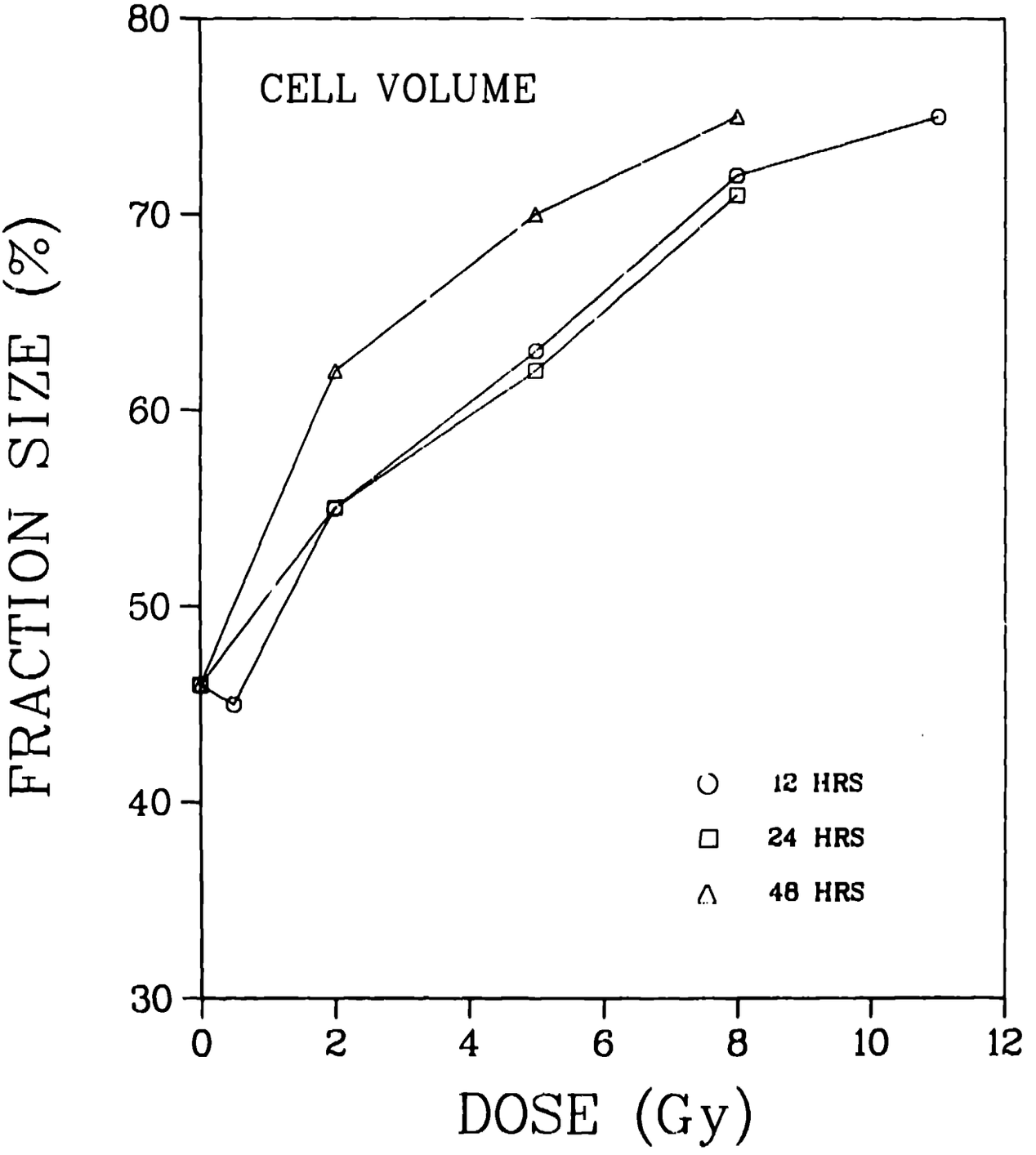
Figure 5. Propidium iodide staining characteristics of peritoneal cells taken 0 to 8 days after a single dose of 5 Gy.

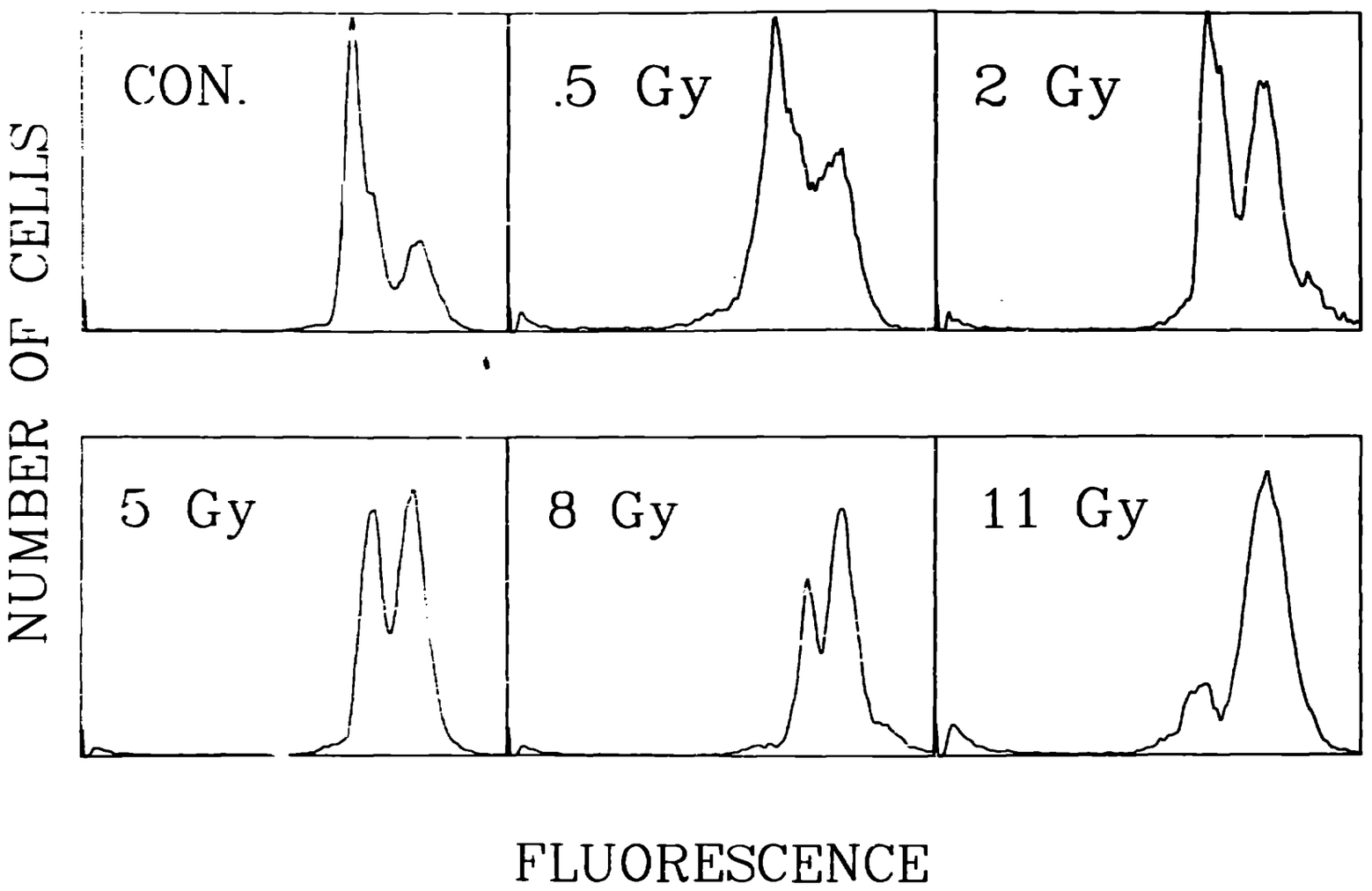
Figure 6. The fraction of cells in the high fluorescence intensity peak as a percent of the total population plotted as a function of dose.

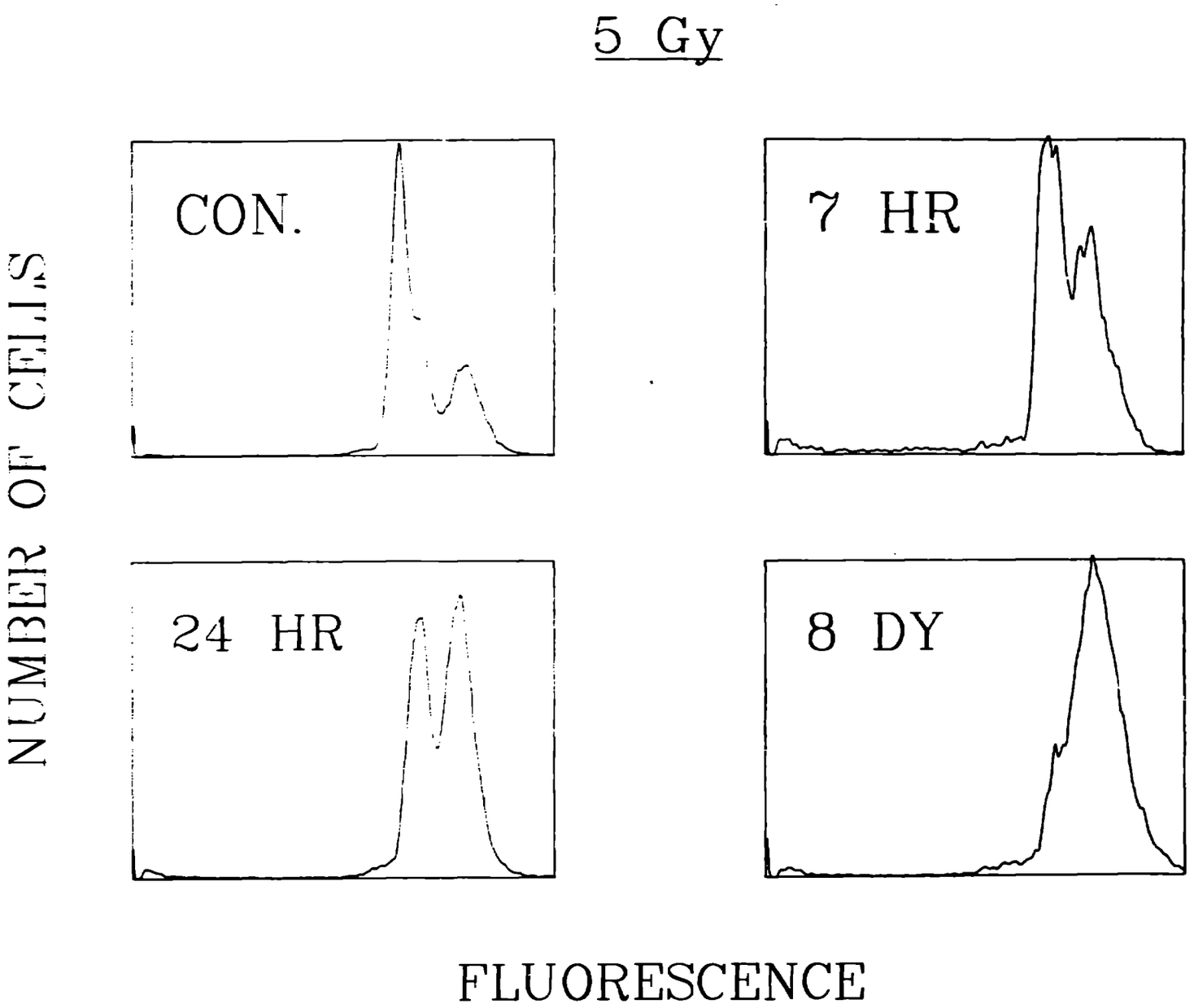
12 HR

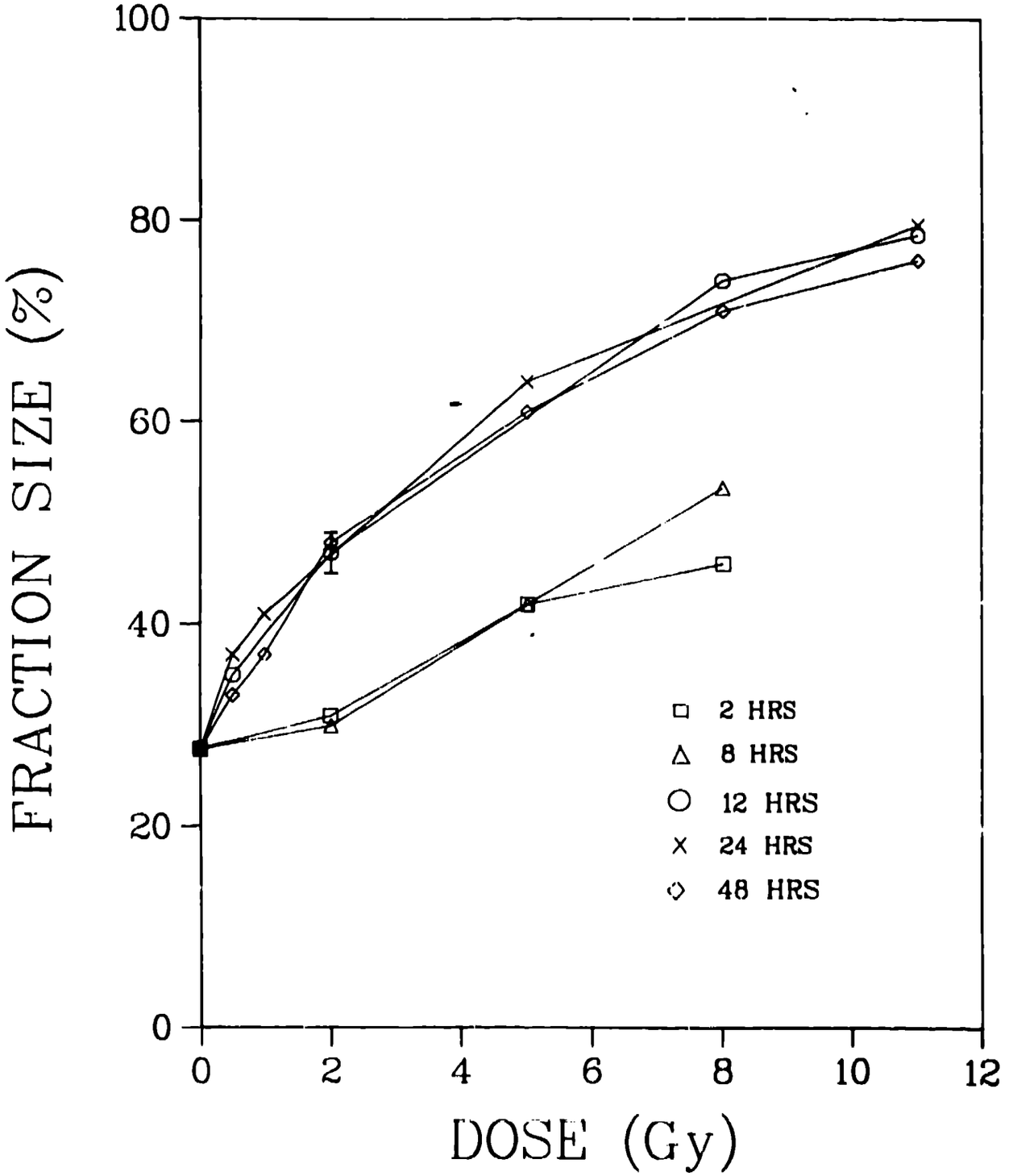



24 HR
C
2 Gy
5 Gy
8 Gy
NUMBER OF CELLS
COULTER VOLUME


CELL VOLUME
FRACTION SIZE (%)
80
70
60
50
40
30
0
2
4
6
8
10
12
DOSE (Gy)
12 HRS
24 HRS
48 HRS


CON.
.5 Gy
2 Gy
5 Gy
8 Gy
11 Gy
NUMBER OF CELLS
FLUORESCENCE


5 Gy
CON.
7 HR
24 HR
8 DY
NUMBER OF CELLS
FLUORESCENCE


100
80
60
40
20
0
FRACTION SIZE (%)
0
2
4
6
8
10
12
DOSE (Gy)
2 HRS
8 HRS
12 HRS
24 HRS
48 HRS